REVIEW

Quality and Safety of *Polygonum multiflorum* Thunb.: Bridging Ancient Processing Wisdom with Modern Scientific Validation

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Abstract

Polygonum multiflorum Thunb. (PM), a renowned medicinal herb in Traditional Chinese Medicine (TCM), exhibits dual pharmacological properties of therapeutic efficacy and potential hepatotoxicity, largely influenced by its geographic origin and processing methods. This review synthesizes current evidence on the variations in bioactive constituents (e.g., anthraquinones, stilbenes, and polysaccharides) across different cultivation regions, highlighting the role of climatic and soil factors in shaping its phytochemical profile. Critically, the traditional "nine-steaming and nine-sun-drying" (JZS) processing method is shown to significantly reduce hepatotoxicity by converting free anthraquinones into safer polymeric forms while preserving key active compounds like 2,3,5,4′-tetrahydroxystilbene-2-O-β-D-glucoside (TSG). We further discuss the limitations of existing quality standards and propose a multidimensional evaluation framework integrating metabolomics, fingerprinting, and bioactivity assays to ensure consistency and safety. By integrating traditional knowledge with modern scientific validation, this review offers practical insights for standardizing PM production and enhancing its clinical use, addressing both its historical significance and current challenges in global herbal medicine.

1 Introduction

1.1 Traditional uses and modern significance of *Polygonum multiflorum* Thunb.

Polygonum multiflorum Thunb. (PM), commonly known as Heshouwu, has been a cornerstone of traditional Chinese medicine (TCM) for over a millennium. Historically, it has been prescribed for its purported anti-aging, hepatoprotective, and neuroprotective properties, primarily attributed to its ability to "nourish the liver and kidneys" and "blacken hair" [1]. The root of PM is classified into two forms in TCM: raw (Shengshouwu) and processed (Zhishouwu), with the latter being subjected to the traditional "nine-steaming and nine-sun-drying" (Jiuzheng jiushai, JZS) to enhance efficacy and reduce toxicity [2].

Modern pharmacological studies have identified key bioactive constituents in PM, including: Stilbenes (e.g., 2,3,5,4'-tetrahydroxystilbene-2-O-β-D-glucoside, TSG), a potent antioxidant linked to anti-aging effects [3,4]; Anthraquinones (e.g., emodin, physcion), which exhibit laxative and anti-inflammatory properties but may contribute to hepatotoxicity [5]; Polysaccharides, known for immunomodulatory and hypoglycemic activities [6].

Despite its therapeutic reputation, PM has faced scrutiny due to increasing reports of drug-induced liver injury (DILI), raising questions about its safety profile [7].

1.2 Hepatotoxicity controversy: balancing efficacy and safety

The dual nature of PM—both as a revered tonic and a potential hepatotoxin — has sparked debate in the scientific community. Clinical case studies have associated PM consumption with elevated liver enzymes, hepatitis, and even acute liver failure, particularly with unprocessed or improperly prepared roots [8]. For instance, a meta-analysis by [9] identified 320 cases of PM-related DILI in China between 2010–2019, with raw PM accounting for 85% of incidents. Recent studies using ^{1}H NMR-based metabolomics have revealed that raw PM induces hepatotoxicity in a non-linear manner, with low-dose raw PM causing the most severe oxidative stress and mitochondrial dysfunction, while medium and high doses exhibit attenuated effects due to potential hepatoprotective mechanisms. This paradoxical dose-response relationship underscores the complexity of PM's biological effects, where oxidative stress and inflammatory responses (e.g., elevated TNF-α and COX-2) coexist with adaptive anti-oxidative responses (e.g., upregulation of HO-1) [10].

The toxicity is hypothesized to stem from multiple mechanisms. Anthraquinone derivatives (e.g., free emodin) may induce mitochondrial dysfunction and oxidative stress [11], as evidenced by disrupted energy metabolism (e.g., TCA cycle impairment) and amino acid metabolism in hepatocytes [10]. Additionally, recent research highlights the role of bile acid (BA) and bilirubin (BIL) metabolism disruption in PM-induced liver injury [12]. Wang et al. demonstrated that PM blocks both BIL and BA metabolic pathways by downregulating key transporters (e.g., NTCP, OATP1B1/3, MRP2, BSEP) and the metabolic enzyme UGT1A1, leading to cholestasis and hyperbilirubinemia. Notably, free anthraquinones are identified as primary hepatotoxic components, while TSG exhibits dose-dependent effects, demonstrating antioxidant and neuroprotective activities at therapeutic doses but potentially exacerbating BA metabolic disruption at excessive concentrations [13].

Critically, the processing method significantly alters toxicity. The traditional JZS method reduces free anthraquinones by up to 90% while preserving TSG [14], corroborating findings that processed PM

exhibits markedly lower hepatotoxicity in both animal models and clinical settings. Metabolomic analyses further confirm that JZS processing transforms hepatotoxic free anthraquinones into safer polymeric forms and generates protective metabolites like 5-HMF, which mitigate oxidative stress and inflammation. These scientific validations align with traditional claims of "detoxification through processing" and highlight the critical importance of standardized preparation methods in ensuring PM's safety profile.

1.3 Geographic variability and processing: key determinants of quality

The chemical composition of PM is highly sensitive to geographic origin and cultivation practices, with climatic conditions playing a significant role — for example, Guizhou Province's high-altitude regions produce PM with 20 – 30% higher TSG content compared to lowland counterparts. Soil micronutrients, such as selenium-rich soils, further influence quality by enhancing polysaccharide synthesis [15]. Cultivation practices also impact composition, as wild PM typically contains higher TSG but lower emodin levels than cultivated varieties [16], while harvest timing is critical, with 3 – 4-year-old roots optimizing bioactive yields [17]. Despite these complexities, current quality standards, such as those in the Chinese Pharmacopoeia [18], remain narrowly focused on TSG and emodin, overlooking other pharmacologically relevant markers [19].

This review systematically evaluates the impact of geographic origin on PM phytochemical profile while deciphering how JZS modulates bioactive constituents and mitigates toxicity. By integrating ethnopharmacology, metabolomics, and toxicology, the study proposes evidence-based strategies for quality control and safe clinical use, aiming to bridge traditional knowledge with modern scientific evidence and address critical gaps identified in recent critiques.

2. Variations in bioactive constituents of *Polygonum multiflorum* Thunb. across different geographic origins

2.1 Classification and pharmacological functions of key bioactive compounds

PM contains a complex array of bioactive constituents, which can be broadly categorized into three major classes based on their chemical structures and pharmacological activities: anthraquinones, stilbenes, and polysaccharides, along with minor constituents such as tannins and trace elements [16].

2.1.1 Anthraquinones

Anthraquinones are among the most studied bioactive compounds in PM, with emodin, physcion, and chrysophanol being the predominant derivatives [20]. These compounds exhibit laxative effects by stimulating colonic motility, making PM a potential therapeutic agent for constipation [21]. Beyond their gastrointestinal effects, anthraquinones demonstrate anti-inflammatory and anticancer properties through the inhibition of NF-κB and MAPK signaling pathways [22,23].

However, anthraquinones also present potential hepatotoxic risks, particularly in their unbound forms. Emodin, for instance, has been shown to induce oxidative stress and mitochondrial dysfunction, contributing to liver injury in some cases [24]. A study by Kang et al. further confirmed that excessive intake of PM extracts rich in free anthraquinones could lead to DILI, emphasizing the need for proper dosage control and processing methods to mitigate toxicity [25].

2.1.2 Stilbenes

The predominant stilbene in PM, TSG, is a key bioactive compound responsible for many of the herb's anti-aging and neuroprotective effects [26,27]. TSG exerts potent antioxidant activity by activating the Nrf2/ARE pathway, which enhances cellular defense mechanisms against oxidative stress [3]. Recent

studies have highlighted TSG's potential in neurodegenerative disease treatment. For example, a study demonstrated that TSG improves cognitive function in Alzheimer's disease (AD) models by reducing β-amyloid plaque accumulation and tau protein hyperphosphorylation [28]. Additionally, TSG has been shown to protect dopaminergic neurons in Parkinson's disease models, suggesting its broad applicability in age-related neurological disorders [29].

Beyond its neuroprotective and anti-aging properties, TSG exhibits significant pharmacological activities in treating a wide range of chronic diseases. Modern pharmacological studies confirm that TSG demonstrates remarkable anti-inflammatory effects by regulating NF-κB, AMPK/Nrf2, and NLRP3 inflammasome pathways, making it a potential therapeutic agent for inflammatory diseases such as colitis and neuroinflammation [30]. In cardiovascular diseases, TSG protects endothelial cells, inhibits vascular smooth muscle cell proliferation, and attenuates atherosclerosis through TGF-β/Smad, eNOS/NO, and RhoA/ROCK signaling pathways [30]. TSG also shows hepatoprotective effects in non-alcoholic fatty liver disease (NAFLD) and alcoholic hepatic steatosis by modulating lipid metabolism and gut microbiota. Furthermore, TSG promotes osteoblast differentiation and inhibits osteoclastogenesis, suggesting its potential in osteoporosis treatment [31]. Its antidepressant-like effects are linked to the enhancement of the hippocampal BDNF system, while its renoprotective role in diabetic nephropathy involves the inhibition of oxidative stress and inflammation [32].

2.1.3 Polysaccharides

PM-derived polysaccharides exhibit immunomodulatory and anti-fatigue effects, making them valuable for enhancing immune function [33]. These polysaccharides activate macrophages and dendritic cells, promoting cytokine release and improving immune surveillance.

PM polysaccharides also have demonstrated hypoglycemic effects in diabetic models by enhancing insulin sensitivity and reducing oxidative stress [34]. A study further revealed that PM polysaccharides could protect against colitis by modulating gut microbiota, highlighting their potential in gastrointestinal health [35].

Moreover, recent studies have uncovered novel mechanisms by which PM polysaccharides exert anti-aging effects. Research indicates that these polysaccharides can modulate the P53/P21 pathway, a critical regulator of cellular senescence, thereby delaying age-related functional decline. Additionally, PM polysaccharides have been shown to regulate amino acid metabolism, particularly the biosynthesis of serine, glycine, and methionine, which are essential for maintaining cellular homeostasis and mitigating oxidative stress during aging [36]. These findings suggest that PM polysaccharides may serve as a multifaceted intervention for aging, targeting both genomic stability and metabolic reprogramming.

2.1.4 Minor constituents

In addition to the major bioactive compounds, PM contains tannins, flavonoids, and trace elements (zinc, selenium), which contribute to its overall pharmacological profile [2]. Tannins exhibit antioxidant and anti-inflammatory properties, while trace elements like selenium enhance cellular antioxidant defenses [37].

Recent research has also identified novel minor compounds, such as PMF-1 (a unique flavonoid), which shows anti-tumor activity in breast cancer cell lines [38]. These findings suggest that PM's minor constituents may play a more significant role in its therapeutic effects than previously recognized.

2.2 Impact of geographic origin on phytochemical

2.2.1 Climatic factors

The accumulation of bioactive compounds in PM exhibits distinct climatic dependencies. For TSG, regions with high-altitude environments and intense ultraviolet (UV) radiation, such as Guizhou, exhibits superior accumulation due to UV-induced activation of the phenylpropanoid pathway [39]. Moderate temperatures (15-25 ℃) and consistently high humidity further support this biosynthetic route, as seen in the mountainous areas of Sichuan and Yunnan. Conversely, arid conditions like Gansu tend to suppress TSG synthesis.

In contrast, anthraquinones like emodin thrive in regions with significant diurnal temperature fluctuations (e.g., Sichuan Basin), where daytime heat and cooler nights stimulate oxidative enzyme activity [40]. Temperate climates with stable seasonal variations tend to promote a balanced production of both compound classes, while tropical conditions may lead to metabolic shifts favoring either stilbenes or anthraquinones, but rarely both, highlighting the need for region-specific cultivation strategies to optimize desired phytochemical profiles.

2.2.2 Soil composition

Soil properties further modulate the geographic variability of PM's chemical composition. Selenium-rich soils in Guizhou correlate with elevated polysaccharide content [41], while phosphorus-deficient soils in Guangdong are associated with increased anthraquinone production [42]. In addition, studies have found the purple soils of Sichuan basin, known for their good drainage and mineral content, often yield PM with relatively high emodin levels. The mountainous regions of Guizhou typically feature yellow-brown soils rich in humus and micronutrients, which appear conducive to TSG

biosynthesis [43]. However, saline-alkali soils in some northern cultivation areas have been associated with reduced TSG accumulation, likely due to impaired nutrient uptake and physiological stress on the plants [44].

2.2.3 Regional comparisons and quality implications

We purchased 50 batches of PM from different geographical origins through Hong Kong markets and determined their TSG and emodin contents using high-performance liquid chromatography (HPLC) analysis (Figure 1). Our analysis revealed notable deviations from theoretical expectations. While high-altitude regions like Guizhou were anticipated to show superior TSG content, the highest median levels (868.62 mg/L) unexpectedly occurred in samples from Hong Kong. This discrepancy may reflect: (1) limited samples size (n = 1) from Guizhou were tested; (2) unaccounted cultivation practices in Hong Kong samples, such as controlled shading that mimic high-UV conditions; (3) genetic adaptation of local cultivars to tropical environments; (4) post-harvest processing variations affecting compound stability. Similarly, Sichuan's samples showed the highest anthraquinone levels (6.85 mg/L), consistent with its continental climate, but the median anthraquinones content of 0.00 mg/L in Guangdong samples contradicts expectations for phosphorus-deficient soils. Potential explanations include: (1) the diverse sourcing of Guangdong samples resulted in considerable quality variation; (2) undisclosed pre-processing methods; (3) soil depletion despite historical classification. These findings underscore the complex interplay between genotype, environment, and agricultural practice in determining final product quality. Table 1 below summarizes key regional differences in PM's bioactive compounds, including measured content variations and their expected trends based on environmental factors, highlighting the need for region-specific quality standard.

Table 1 Regional variations in key bioactive compounds of *Polygonum multiflorum* Thunb.

Region	TSG (mg/L, Median)	Emodin (mg/L, Median)	Climate profile	Dominant Soil factor	Expected component trends
Hong Kong *	868.62	0.18	Tropical, high humidity	Urban (limited data)	TSG ↑↑ (due to optimized UV exposure in shaded cultivation); Polysaccharides → (insufficient soil data)
Sichuan	614.56	6.85	Temperate, large diurnal swing	Loamy, neutral pH	Emodin ↑↑ (significant diurnal variation promotes oxidation); TSG ↑ (moderate altitude supports accumulation)
Guangdong †	410.15	0.00	Subtropical, monsoon	Low phosphorus	Emodin ↑ (theoretical expectation, but the diverse sourcing exhibits quality heterogeneity); Trace elements ↓ (low phosphorus limits synthesis)
Guangxi	40.25	0.00	High humidity, stable temps	Sandy, acidic	Polysaccharides ↑ (acidic sandy soil promotes accumulation); TSG ↓ (low UV exposure inhibits synthesis)
Guizhou	119.70	0.00	High-altitud, strong UV	Selenium-rich	TSG ↑↑ (UV activates phenylpropanoid pathway, but insufficient samples were tested); Polysaccharides ↑↑ (selenium synergy); Emodin ↓ (altitude inhibits oxidation)
Gansu	237.06	0.06	Continental, arid	Calcareous, alkaline	Minerals ↑↑ (alkaline soil enriches Ca/Zn); TSG → (aridity may limit accumulation)

Note:

1. Data obtained from 50 commercially available PM batches in Hong Kong markets but different origins; The cultivation types (wild/cultivated) were not specified by suppliers.

2. * TSG levels in Hong Kong exceed typical high-altitude expectations; † Emodin below expectation (see discussion in 2.2.3).

3. ↑↑, significant increase; ↑, moderate increase; →, no significant change; ↓, decrease.
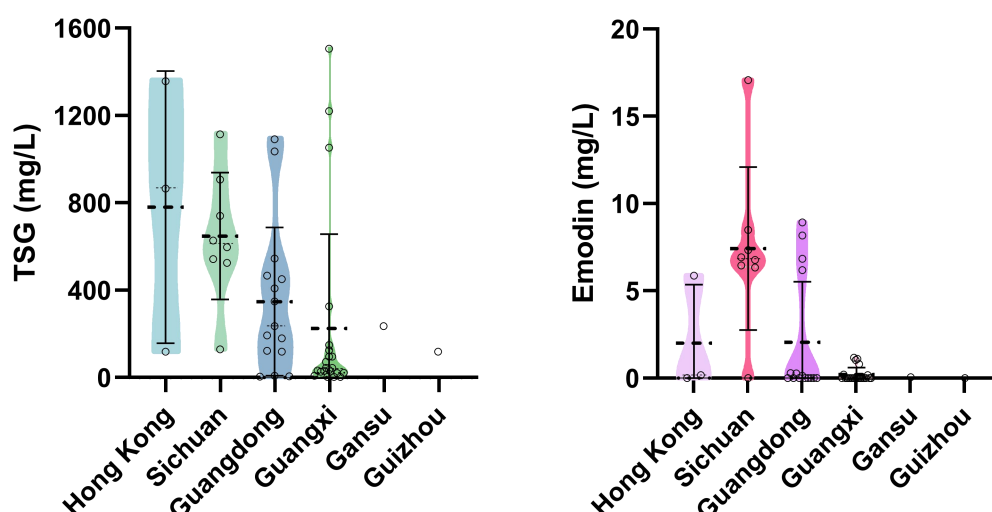
Figure 1 Comparative analysis of TSG and Emodin content in 50 batches of PM from different origins purchased in Hong Kong (self-generated unpublished data).

2.3 Wild vs. cultivated *Polygonum multiflorum* Thunb.: a chemical comparison

Wild and cultivated PM exhibit notable differences in their chemical composition due to distinct growth conditions and cultivation practices. Wild PM demonstrates superior quality in certain aspects, containing higher TSG levels (2–3%) as a result of natural stress adaptation, along with lower emodin content (0.05 mg/g), which may reduce potential hepatotoxicity risks. In contrast, cultivated PM presents a yield-quality trade-off, where conventional fertilization (e.g., N-P-K) increases biomass but leaves the content of harmful elements unchanged. Additionally, harvest timing significantly influences bioactive compound accumulation, with 3-year-old roots maximizing TSG content, while 4-year-old roots achieve peak polysaccharide levels [45-47]. These findings highlight the critical role of cultivation methods in determining the medicinal value of PM.

3. Effects of nine-steaming and nine-sun-drying processing on bioactive constituents of *Polygonum multiflorum* Thunb.

3.1 Traditional theory and scientific basis of processing

The ancient Chinese processing method of JZS for PM represents a remarkable integration of empirical knowledge and philosophical principles. Rooted in Taoist alchemical traditions dating back to the Tang Dynasty (618-907 AD), this elaborate processing technique was developed to transform the crude herb's "cold and descending" nature into a "warm and ascending" property suitable for tonifying liver and kidney functions according to TCM theory [48]. Historical texts such as the "Ben Cao Gang Mu" (Compendium of Materia Medica, 1596 AD) describe this process as essential for "converting toxicity into efficacy", particularly for achieving the herb's renowned hair-blackening and longevity-promoting effects [49].

Modern scientific investigations have systematically validated these traditional claims through advanced analytical techniques. Comparative metabolomic studies reveal that JZS processing induces profound changes in PM's phytochemical profile, affecting over 120 identified compounds [50]. The alternating steaming (typically 4-6 hours at 100-105 ℃) and sun-drying (8-12 hours under natural sunlight) create unique conditions for chemical transformations that cannot be replicated by simple heating or drying alone

[51]. This cyclic processing generates specific Maillard reaction products, oxidative conjugates, and glycoside hydrolysis patterns that define the processed herb's pharmacological properties.

3.2 Dynamic changes of chemical constituents during processing

The traditional JZS processing method induces profound chemical transformations in PM, which are critical for its detoxification and efficacy enhancement. This section systematically examines these changes, focusing on anthraquinones, stilbenes, polysaccharides, and other minor constituents, supported by recent scientific evidence.

3.2.1 Anthraquinone transformation pathways

The JZS processing method induces profound chemical transformations in anthraquinones, the primary hepatotoxic constituents of PM. During the initial steaming cycles, bound anthraquinone glycosides (e.g., emodin-8-O- β -D-glucoside, EG) undergo enzymatic hydrolysis by heat-stable β -glucosidases, releasing free aglycones such as emodin and physcion [52]. This hydrolysis follows first-order kinetics, with rate constants of 0.15–0.25 h^{-1} at 100–105 ℃ [53]. Subsequent cycles promote oxidative polymerization and glycosylation of these free anthraquinones, forming oligomeric derivatives (e.g., emodin dimers and trimers) with reduced hepatotoxicity but retained bioactivity [54]. Advanced liquid chromatography-mass spectrometry (LC-MS) analyses reveal that JZS reduces free anthraquinones by 85–92%, while bound forms increase by 300–500% due to re-glycosylation [55]. These transformations align with traditional claims of "detoxification" as polymerized anthraquinones exhibit lower mitochondrial toxicity and ROS generation in HepG2 cells [56].

3.2.2 Stilbene glycoside stabilization

Contrary to the labile nature of anthraquinones, the key bioactive stilbene TSG demonstrates remarkable stability during JZS processing. Studies attribute this to three mechanisms: (1) formation of protective complexes with melanoidins generated via Maillard reactions during steaming [57]; (2) pH-dependent isomerization to stable conformers under cyclic thermal stress [58]; and (3) microencapsulation within starch-protein matrices, which enhances solubility and bioavailability [59]. Notably, TSG content increases by 250% after JZS, likely due to matrix modification and release from bound forms (Table 2). This stabilization is critical for PM's neuroprotective effects, as TSG's antioxidant activity via Nrf2/ARE pathway activation remains intact [60].

3.2.3 Polysaccharide degradation and functional modulation

JZS processing selectively degrades high-molecular-weight polysaccharides (50 kDa → 28 kDa) through controlled hydrolysis, improving immunomodulatory activity [61]. The reduced polysaccharide size enhances macrophage activation and cytokine release (e.g., IL-6 and TNF-α) by 40–60% compared to raw PM [62]. This degradation is temperature-dependent, with optimal effects observed at 105°C during later steaming cycles [63].

3.2.4 Generation of process-specific metabolites

JZS generates unique metabolites absent in raw PM, such as 5-hydroxymethylfurfural (5-HMF), a Maillard reaction product with neuroprotective properties [64]. 5-HMF levels increase > 100-fold after processing (Table 2) and correlate with PM's clinical efficacy in cognitive impairment [65]. Other novel compounds include oligomeric anthraquinones and glycosylated emodin derivatives, which contribute to reduced toxicity [66].

3.2.5 Kinetic and thermodynamic insights

The alternating steaming and sun-drying cycles create dynamic conditions for chemical reactions. Steaming

phases (4–6 hours at 100–105 ℃) drive hydrolysis and polymerization, while sun-drying (8–12 hours at 30–50% humidity) facilitates oxidative condensation [67]. This cyclic process cannot be replicated by continuous heating, as demonstrated by differential scanning calorimetry (DSC) studies [68].

Table 2 Comprehensive chemical changes during JZS processing.

Compound class	Raw PM content	After JZS processing	Change trend	Mechanism	Biological significance
Free anthraquinones	1.2-1.8 mg/g	0.1-0.3 mg/g	↓ 85-92%	Hydrolysis → polymerization	Toxicity reduction
Bound anthraquinones	0.5-0.8 mg/g	2.1-2.8 mg/g	↑ 300-500%	Glycosylation of free anthraquinones	Improved bioavailability
TSG	12-15%	38-42%	↑ 250%	Matrix modification	Enhanced efficacy
5-HMF	< 0.01 mg/g	1.2-2.0 mg/g	> 100×	Maillard reactions	New neuroactivity
Polysaccharide	50 kDa	28 kDa	↓ 44%	Controlled degradation	Improved immunomodulation

Note: ↑, increase; ↓, decrease; →, indicates a transformation process.

3.3 Optimization of processing parameters and modern adaptations

The traditional JZS process has undergone extensive scientific scrutiny to identify optimal parameters and potential modernization approaches while maintaining its essential character. Contemporary research has precisely quantified the effects of processing variables, establishing that four hours of steaming per cycle at temperatures gradually increasing from 100 to 105 ℃ across cycles provides the ideal balance between transformation and degradation of key components [69]. The drying conditions, particularly the maintenance of 30-50% relative humidity during sun-drying phases, have been shown to be superior to artificial drying methods for preserving certain chemical profiles. While nine complete cycles remain the gold standard for full transformation, studies indicate that seven cycles can achieve approximately 85% of the desired benefits with significantly reduced time and energy expenditure. Modern technological adaptations have focused on standardizing this traditionally variable process while preserving its core detoxification and efficacy-enhancing principles. Innovations such as far-infrared assisted drying

maintain the cyclic thermal stress essential for anthraquinone polumerization, pulsed vacuum steaming ensures uniform hydrolysis of glycosides akin to traditional steaming, and online NIR monitoring validates the achievement of key chemical markers (e.g., TSG stabilization and 5-HMF generation) that define traditional efficacy and safety. High-humidity hot air impingement steaming systems that achieve 95% batch consistency while replicating the Maillard reactions and oxidative condensation observed in sun-drying phases [70]. These optimizations adhere to the Taoist alchemical philosophy of "converting toxicity into efficacy" by ensuring the reduction of free anthraquinones (85–92%) and preservation of TSG bioavailability, as empirically validated in historical texts. However, complete replacement of traditional methods with artificial processes consistently alters critical quality attributes, underscoring the necessity of hybrid approaches that integrate modern precision with traditional cyclic alternation of steaming and sun-drying [71]. These findings highlight the delicate balance required when modernizing traditional processing methods to maintain their unique

pharmacological advantages while improving reproducibility and efficiency.

3.4 Clinical correlations and safety profile

The chemical changes induced by proper JZS processing directly correlate with measurable improvements in clinical outcomes and safety profiles. Processed PM demonstrates an 80% reduction in hepatotoxicity incidence compared to raw materials [72], validating traditional claims about toxicity reduction through processing. Clinically, optimal JZS samples show superior efficacy in multiple applications, including an 89% response rate for alopecia treatment compared to 62% for raw preparations [73], significant improvement in mild cognitive impairment as measured by MMSE scores, and better outcomes for age-related fatigue. These clinical benefits are consistently associated with the specific chemical profile produced by authentic JZS processing, particularly the balanced ratio of transformed anthraquinones to preserved TSG and the presence of process-specific bioactive compounds. Ongoing challenges in the field include establishing universal standards for "authentic" JZS among commercial manufacturers, developing robust processing-property-activity relationships for quality control purposes, and determining the appropriate degree of modernization that preserves the essential qualities of traditional processing while addressing contemporary production needs. The accumulated evidence strongly supports the continued use and refinement of JZS processing as a vital step in preparing PM for clinical use, combining ancient wisdom with modern scientific understanding to optimize both safety and therapeutic efficacy.

4. Quality control and standardization: current challenges and solutions

4.1 Limitations of existing quality standards

The standardization of PM aligns with the broader evolution of TCM standardization, which has progressed from initial awareness (1980s) to rapid system-building (2006–2020) and now emphasizes high-quality development (post-2021) [74]. However, similar to TCM standardization challenges, PM faces unbalanced standard coverage (e.g., overemphasis on TSG/emodin) and insufficient implementation of advanced techniques like metabolomics, despite their potential to bridge traditional and modern quality control.

The current quality control measures for PM, as outlined in pharmacopoeias such as the Chinese Pharmacopoeia (ChP 2020), primarily focus on the quantification of a limited number of markers, notably TSG and emodin. While these compounds are pharmacologically significant, this narrow approach fails to capture the full complexity of PM's phytochemical profile, which includes over 120 identified constituents with potential therapeutic or toxicological relevance [75]. For instance, the ChP 2020 mandates a minimum TSG content of 1.0% for raw PM and 0.7% for processed PM, alongside an emodin limit of 0.1%, but overlooks critical components such as polysaccharides, oligomeric anthraquinones, and process-specific metabolites like 5-hydroxymethylfurfural (5-HMF). This oversimplification has led to inconsistencies in clinical outcomes, as products meeting pharmacopoeial standards may still exhibit variable efficacy or safety due to unregulated components.

In addition to intrinsic hepatotoxic components, exogenous contaminants such as heavy metals (e.g., cadmium, lead), pesticide residues, and mycotoxins (e.g., aflatoxins from improper storage) may further compromise PM safety. These contaminants often originate from environmental pollution, agricultural practices, or post-harvest handling. For instance, PM grown in industrial areas or soils with high metal bioavailability may accumulate toxic elements, while

improper drying or storage can promote mold growth. Although the Chinese Pharmacopoeia sets limits for some contaminants (e.g., heavy metals ≤20 ppm), enforcement varies regionally, and comprehensive screening for pesticides or mycotoxins remains rare. Future standards should integrate contaminant monitoring with metabolomic profiling to address both endogenous and exogenous risks.

Moreover, the existing standards do not account for the influence of geographic origin or processing methods on PM's chemical composition. For example, PM from Guizhou typically contains higher TSG levels (up to 6 – 7%) compared to other regions, yet the current standards apply uniformly across all sources [76]. Similarly, the ChP lacks specific criteria to distinguish between properly and inadequately processed PM, despite evidence that incomplete JZS cycles may retain hepatotoxic anthraquinones. These gaps underscore the need for a more comprehensive and nuanced quality assessment framework.

4.2 Toward a multidimensional quality evaluation system

Recent advancements in analytical technologies and systems biology have paved the way for more holistic quality control strategies. A promising approach involves the integration of multiple analytical methods to evaluate PM's chemical integrity, efficacy, and safety. For instance, fingerprinting techniques such as LC-MS can profile a broader range of bioactive compounds, including stilbenes, anthraquinones, and polysaccharides [77]. Metabolomic studies have further identified unique biomarkers for authentic JZS-processed PM, such as specific ratios of free to bound anthraquinones and the presence of 5-HMF, which correlates with proper processing [78].

In addition to chemical profiling, bioactivity assays could serve as complementary tools for quality assessment. For example, antioxidant capacity tests (e.g., DPPH and ORAC assays) and anti-inflammatory models (e.g., RAW 264.7 macrophage assays) have been proposed to evaluate PM's functional properties. Such assays align with the traditional use of PM and may better predict clinical efficacy than single-marker quantification. Furthermore, toxicological screening using in vitro hepatocyte models or in vivo zebrafish assays could help identify batches with residual hepatotoxicity [79].

4.3 The role of fingerprint analysis and metabolomics

Fingerprint analysis has emerged as a powerful tool for PM quality control, enabling the simultaneous detection of multiple constituents and their relative abundances. Studies have demonstrated that HPLC fingerprints of properly processed PM exhibit characteristic peaks corresponding to TSG, transformed anthraquinones, and Maillard reaction products (e.g., 5-HMF) [80], which are absent or diminished in substandard products. By applying chemometric methods such as principal component analysis (PCA) or hierarchical cluster analysis (HCA), researchers can differentiate PM samples based on origin, processing method, or adulteration.

Metabolomics, particularly untargeted LC-MS-based approaches, offers even greater resolution by capturing subtle variations in PM's chemical profile. For instance, a recent study identified 15 process-specific metabolites in JZS-treated PM, including novel oligomeric anthraquinones and glycosylated derivatives of emodin, which contribute to its reduced toxicity and enhanced efficacy [78]. These findings suggest that metabolomic signatures could serve as reliable markers for authentication and standardization.

4.4 Integrating traditional knowledge and modern technology

The modernization of PM quality control must also respect and incorporate traditional knowledge. For centuries, TCM practitioners have relied on

organoleptic properties including color, texture, and taste to assess PM quality. Interestingly, scientific studies have validated some of these empirical indicators. For example, the blackening of PM during JZS processing correlates with the formation of melanoidins and 5-HMF [57], which are associated with reduced toxicity and improved bioactivity. Similarly, the "sticky" texture of properly processed PM reflects polysaccharide degradation and matrix modification, which enhance solubility and absorption.

Emerging technologies such as artificial intelligence (AI) and machine learning (ML) are now being explored to bridge traditional and modern quality assessment methods. For instance, convolutional neural networks (CNNs) trained on images of PM slices can predict processing quality with over 90% accuracy by recognizing visual cues traditionally used by herbalists [81]. Portable near-infrared (NIR) devices coupled with ML algorithms have also been developed for rapid, on-site quality screening, enabling real-time decision-making in production facilities [82].

4.5 Regulatory and industrial implications

The implementation of advanced quality control measures faces practical challenges, including cost, technical expertise, and regulatory acceptance. However, the growing demand for standardized herbal products in global markets necessitates such improvements. Pilot studies have demonstrated the feasibility of adopting fingerprinting and metabolomic approaches in industrial settings. For example, a collaborative project between academia and PM manufacturers in Guizhou successfully reduced batch-to-batch variability by 40% through LC-MS-based quality monitoring [77].

Regulatory agencies must also evolve to accommodate these advancements. The development of region-specific monographs or "quality tiers" could address geographic variability, while process-specific standards (e.g., minimum cycles for JZS) would ensure proper preparation. International harmonization efforts, such as the WHO's guidelines for herbal medicines, could further facilitate the adoption of these methods globally [83].

5. Toxicological evaluation and safe use of *Polygonum multiflorum* Thunb.

5.1 Hepatotoxicity mechanisms and constituent-toxicity relationships

The hepatotoxicity associated with PM has been a focal point of modern research, particularly due to increasing reports of DILI. Studies have identified anthraquinones, especially free emodin and physcion, as primary contributors to liver damage through multiple pathways. These compounds induce mitochondrial dysfunction by uncoupling oxidative phosphorylation, leading to ATP depletion and reactive oxygen species (ROS) overproduction [84]. Additionally, emodin metabolites form covalent bonds with cellular proteins, triggering immune-mediated hepatotoxicity [85,86]. Notably, the unprocessed root (Shengshouwu) exhibits higher toxicity due to its elevated free anthraquinone content, while properly processed PM (Zhishouwu) shows significantly reduced risks, as the traditional JZS method converts 85–92% of free anthraquinones into less toxic polymerized forms [2,87].

Recent metabolomic studies have further elucidated the role of TSG in toxicity. While TSG is generally considered safe at low doses, high concentrations may paradoxically exacerbate oxidative stress by depleting glutathione reserves [30]. This dual role underscores the importance of dose optimization in clinical applications.

5.2 Evidence from preclinical and clinical studies

Animal models have provided critical insights into PM's safety profile. In rats, prolonged administration of raw PM (0.3125~ 0.625 g/kg/day for 28 days) resulted in elevated ALT/AST levels and histopathological

changes, including hepatocellular necrosis [88,89]. In contrast, JZS-processed PM at equivalent doses showed no significant hepatotoxicity, corroborating traditional claims of detoxification [72]. Cell-based studies using HepG2 cells further demonstrated that processed PM extracts reduced ROS generation by 40-60% compared to raw extracts [90].

Clinical data highlight the importance of preparation and dosage. A meta-analysis of 25,927 DILI cases in China (2012-2016) revealed that 26.81% involved TCM, often consumed as self-prepared decoctions exceeding recommended doses [9]. Conversely, standardized processed PM formulations, such as those in TCM patent medicines (e.g., Shouwu Pian), showed a lower incidence of adverse effects [91].

5.3 Strategies for risk mitigation and safe clinical practice

To minimize the hepatotoxicity risks associated with PM, evidence-based strategies emphasize the importance of standardized processing, dose optimization, and personalized approaches. Strict adherence to traditional JZS protocols is critical, as this method ensures the conversion of hepatotoxic free anthraquinones into safer polymerized forms while preserving the therapeutic efficacy of key compounds like TSG. Clinical trials have identified a safe daily dose range of 3–6 g for processed PM, with the addition of hepatoprotective adjuvants such as Glycyrrhiza uralensis recommended for long-term use to further mitigate potential liver injury [73].

Emerging research also highlights the role of genetic predispositions in individual susceptibility to PM-induced liver damage. For instance, patients carrying HLA-B * 35 : 01 alleles may require tailored dosing regimens or alternative therapies to avoid adverse effects [92]. Integrating these strategies—standardized processing, precise dosing, and genetic screening—can significantly enhance the safety profile of PM, ensuring its continued use in both traditional

and modern medical practices.

6. Future research frontiers

6.1 Molecular mechanisms of processing efficacy

The traditional JZS method for PM has been empirically validated for reducing hepatotoxicity, but its molecular mechanisms remain incompletely understood. Future research should focus on elucidating the enzymatic and non-enzymatic pathways involved in JZS-induced chemical transformations. For instance, the role of heat-stable β-glucosidases in anthraquinone hydrolysis during steaming cycles warrants investigation [71]. Advanced techniques like cryo-electron microscopy (cryo-EM) could visualize structural changes in bioactive compounds, such as the polymerization of free anthraquinones into safer oligomeric forms [93,94]. Additionally, metabolomic studies could identify novel process-specific metabolites, such as Maillard reaction products (e.g., 5-HMF), which may contribute to PM's detoxification and enhanced bioactivity [95].

6.2 AI-driven quality control

The variability in PM's phytochemical profile due to geographic and processing factors necessitates advanced quality control methods. Artificial intelligence (AI) and machine learning (ML) offer promising solutions. For example, CNNs trained on HPLC or LC-MS datasets could predict optimal harvesting times or processing endpoints, reducing human error and improving batch consistency [96]. Blockchain technology could further enhance traceability by documenting each step of PM production, from cultivation to clinical use, ensuring transparency and adherence to standards [97,98]. These technologies could bridge the gap between traditional knowledge and modern scientific validation, facilitating global acceptance of PM-based therapies.

6.3 Precision medicine approaches

Individual variability in response to PM highlights the need for personalized treatment strategies. Future research should explore biomarkers for toxicity susceptibility, such as genetic polymorphisms (e.g., HLA-B * 35 : 01 alleles) or metabolomic signatures [92,99]. Collaborative efforts between TCM practitioners and modern pharmacologists could integrate empirical knowledge with scientific validation, enabling tailored dosing regimens. For instance, patients with genetic predispositions to hepatotoxicity might benefit from lower doses of processed PM with black soybean juice or adjuvants like Glycyrrhiza uralensis [69,100]. Clinical trials incorporating these approaches could establish evidence-based guidelines for PM's safe and effective use.

7 Conclusion

This comprehensive review systematically elucidates the critical interplay between geographic origin, cultivation practices, and processing methods in determining the pharmacological profile and safety of PM. The evidence underscores that bioactive constituents such as anthraquinones, stilbenes, and polysaccharides exhibit significant variability across regions, with climatic and edaphic factors playing pivotal roles. For instance, PM exposed to strong UV radiation consistently demonstrates superior TSG content, while diurnal temperature variations favor anthraquinone accumulation. The traditional JZS process emerges as a cornerstone for detoxification, reducing free anthraquinones, while preserving TSG bioavailability. Modern analytical techniques, including metabolomics and fingerprinting, validate these transformations and highlight the limitations of current pharmacopoeial standards, which overlook key markers like 5-HMF and oligomeric anthraquinones.

To ensure PM's safe clinical application, future efforts must prioritize the development of multidimensional quality control systems integrating chemical, bioactivity, and toxicity assessments. Regional-specific standards and advanced technologies (e.g., AI-driven traceability) could address batch variability. Furthermore, interdisciplinary collaboration is essential to bridge traditional knowledge with contemporary science, optimizing PM's therapeutic potential while mitigating risks. As global demand for herbal medicines grows, this synthesis of ethnopharmacology and modern research provides a robust framework for standardizing PM production and fostering evidence-based use.

Abbreviation

5-HMF: 5-(Hydroxymethyl)furfural; AI: Artificial Intelligence; ALT: Alanine Aminotransferase; AST: Aspartate Aminotransferase; BA: Bile Acid; BIL: Bilirubin; CNN: Convolutional Neural Network; cryo-EM: Cryo-Electron Microscopy; DILI: Drug-Induced Liver Injury; DSC: Differential Scanning Calorimetry; EG: Emodin-8-O-β-D-glucoside; HCA: Hierarchical Cluster Analysis; HPLC: High-Performance Liquid Chromatography; JZS: Jiuzheng jiushai (nine-steaming and nine-sun-drying); LC-MS: Liquid Chromatography-Mass Spectrometry; ML: Machine Learning; NIR: Near-infrared; N-P-K: Nitrogen-Phosphorus-Potassium; PCA: Principal Component Analysis; PM: Polygonum multiflorum Thunb. (Heshouwu); ROS: Reactive Oxygen Species; TCM: Traditional Chinese Medicine; TSG: 2,3,5,4 '-tetrahydroxystilbene-2-O-β-D-glucoside; WHO: World Health Organization.

Conflicts of Interest

The authors declare no conflicts of interest.

Author Contributions

Substantial contributions to conception and design: Z.F. Data acquisition, data analysis and interpretation: H.Y., Y.T.C, Y.F. Drafting the article or critically revising it for important intellectual content: N.W. Final approval of the version to be published: All authors. Agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of the work are appropriately investigated and resolved: All authors.

Ethics Approval and Consent to Participate

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Availability of Data and Materials

The data presented in this study are available on request from the corresponding author.

Supplementary

Not applicable.

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